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SCANNING ELECTRON MICROSCOPY PROVIDES A NOVEL METHOD TO MAP ABDOMINAL MUSCULATURE IN ARCHAEOGNATHA (INSECTA)

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Scanning Electron Microscopy Provides a Novel Method to Map Abdominal Musculature in Archaeognatha (Insecta). Matushkina, N. — Using scanning electron microscopy, previously overlooked cuticle modifications were revealed in the attachment points of skeletal musculature of *Trigoniophthalmus simplex* (Insecta, Archaeognatha, Machilidae). These findings demonstrate that SEM may serve as an indirect approach for rapidly and precisely mapping complicated abdominal musculature of Archaeognatha. The reference to Archaeognatha is especially relevant for the mapping of the abdominal muscles in insects in the sense of the presence of a hypothesized plesiomorphic sclerite composition in this group of wingless insects.

Key words: cuticle, musculature, SEM, insect morphology, methods.

Introduction

The evolutionary origin and modification of an abdominal ventrum is one of the long-standing issues that remain unresolved in insect morphology. The Archaeognatha, which are the sister group to the Zygentoma and Pterygota, exhibit several distinctive morphological features that differentiate them from other insect orders. Among other, these include distinctive sclerite compositions of abdominal segments in both sexes (Klass & Matushkina, 2012, 2018, Matushkina & Klass, 2020).

Another morphological peculiarity of the skeletomusculature is a system of non-cuticular tendons in Archaeognatha (and Zygentoma), which is absent in Pterygota (Klug

& Klass, 2007). A tentative prerequisite of the complicated abdominal musculature in Archaeognatha is the presence of numerous subdivisions of segmental sclerotisations. Klug & Klass (2007) critically analysed published data on two species of Archaeognatha (by Birket-Smith, 1974; Bitsch, 1973) and concluded that the diversity and variability of the musculature in Archaeognatha may cause homology issues and result in this character system having low phylogenetic informativeness as landmarks in the attempt to homologise sclerites. However, due to the lack of original studies on a larger number of species, we regard these conclusions as preliminary and requiring verification.

Examining the cuticular microstructure with scanning electron microscopy (SEM) provides new insights into several aspects of the structural morphology of Archaeognatha (Matushkina, 2011, 2017; Klass & Matushkina, 2018; Matushkina & Klass, 2020). In the present study, we investigated the inner cuticular surface of the female genital segments of *Trigoniophthalmus* (Archaeognatha, Machilidae) using scanning electron microscopy (SEM). Our results demonstrate that SEM can serve as a powerful supplementary tool for rapidly and precisely mapping abdominal musculature. Thus, routine SEM analysis of cuticular surfaces can reveal morphological patterns both in the skeleton and musculature that would otherwise remain neglected.

Material and Methods

The postabdomen of one adult female of *Trigoniophthalmus simplex* Kaplin, 1999 (Archaeognatha: Machilidae), collected in Abkhazia Region (Georgia) by Mykola Kovblyuk and Mykola Yunakov and stored in 70% ethanol, was separated from the rest of the body, dissected with fine scissors and tweezers, and macerated in 10% KOH at room temperature for 10–12 h. Macerated cuticle parts were rinsed in distilled water, dehydrated through a graded ethanol–acetone series, and critical-point dried (OM CPD 7501). Samples were mounted on stubs, coated with gold-palladium (OM-SC7640), and examined with a Zeiss EVO-50 SEM at the Museum of Zoology, Senckenberg Natural History Collections, Dresden, Germany.

To indicate sclerite composition and muscles of *Trigoniophthalmus simplex*, original data were compared with published data on female *Petrobiellus takunagae* Silvestri, 1943 (Klass & Matushkina, 2012: data on sclerite composition and cuticle miscrosculpture) and *Trigoniophthalmus alternatus* (Silvestri, 1904) (Bitsch, 1974: data on muscles). The following abbreviations were used in Fig. 1: CX (+number) = coxa/−ite (number = segment); LCa9 = antelaterocoxa/−ite of segment 9 (number = segment); LCp9 = postlaterocoxa/−ite of segment 9; PS9 = ‘poststernite’ at ventral hind margin of segment 9; STt (+number) = (‘true’) sternum/−ite (number = segment); TG (+number) = tergum/−ite (number = segment).

Results

The inner (i. e., facing the body cavity) surface of the cuticle of the female postabdomen is composed of flat sclerites, bearing openings of the attachment sites of scales (particularly on coxae and sternal sclerotisations), which are largely separated by more irregularly wrinkled membranes. Well-defined, slightly protruding areas of cuticle

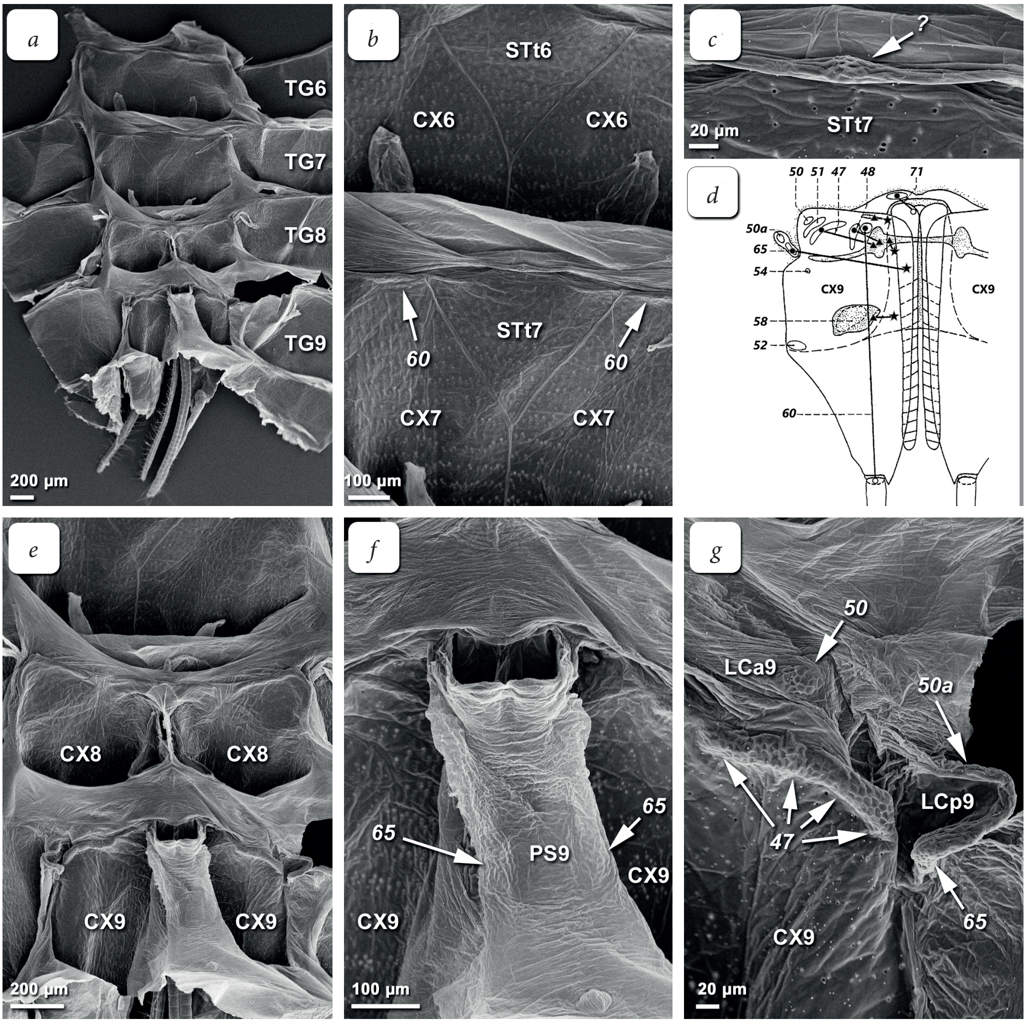

Fig. 1. Attachment points of the abdominal musculature in the female *Trigoniophthalmus* (Archaeognatha: Machilidae), showing on inner cuticular surface of *T. simplex*, SEM (*a–c*, *e–g)* and on schematic representation of ventral musculature of segment 9 in female *T. alternatus* (*d*): *a* — segments 6–9; *b* — ventrum 6 and ventrum 7; *c* — sternal sclerotisations of segment 7; *d* — Fig. 6 from Bitsch, 1974, modified; *e* — ventrum 8 and ventrum 9; *f* — intercoxal cuticle of ventrum 9; *g* — anterolateral margin of ventrum 9. Note that hexagonal microsculpture marks attachment points of muscles; numbers of muscles in italics follow Bitsch (1973, 1974). "?" indicates the attachment of a muscle to intersternite 7 not described in the literature. Abbreviations of abdominal sclerotisations are given in Material and Methods

with hexagonal sculpture (cell size ab. 3–5 μm) are found on sclerites only. The prominence of the hexagonal sculpture varies from very clear (on anterior part of coxae 7, 8, and 9; on antelaterocoxae 9 and postlaterocoxae 9; and on intersternite 7) to weakly discernible from adjacent regions (on the lateral margins of poststernite 9) (Fig. 1).

Discussion

Topographic homology represents the initial approach in the process of homologisation of anatomical structures. Homological muscles are often served as criteria in homologisation of abdominal sclerites in insects, and conversely, attachments to homological sclerites are served as a criterion in homologisation of abdominal muscles (Klug & Klass, 2007). The origins and insertions of muscles were used to clarify the homology of sclerotized structures to which they are attached, e. g., in Lepidoptera (Solis & Metz, 2011), in ants (Lieberman et al., 2022), and across several insect orders (Klass et al., 2012).

Archaeognatha represent an ancient lineage of wingless insects (Song et al., 2019). Their basal position within the insect tree of life, combined with the retention of numerous ancestral traits, makes them pivotal for understanding the early evolution of hexapods. The ventrum of the typical abdominal segment of Archaeognatha retains well-developed bilateral coxites, laterocoxites and medial sternal sclerites that serve as attachment sites for an intricate system of muscles. Several smaller sclerotisations may also be visualized (often using SEM), at least in some body regions, such as the medial intersternite, and poststernite 9 (Klass & Matushkina, 2012, 2018; Matushkina & Klass, 2020). This morphological peculiarity of Archaegnatha contrasts essentially with the more compact and simplified sclerite composition of the abdominal segments in Zygentoma and, even more so, in Pterygota. Moreover, the abdominal architecture of Archaeognatha is functionally linked to their behaviour, conferring the skeletal flexibility and muscular power required for their characteristic jumping locomotion (Sturm & Machida, 2001).

The peculiar sclerite composition of Archaeognatha is closely associated with a distinctive and highly diverse organisation of abdominal musculature. To the best of my knowledge, detailed descriptions of abdominal musculature in Archaeognatha are provided in only two studies. Birket-Smith (1974) documented the abdominal muscles of *Petrobius lohmanderi* Agrell, 1944, and Bitsch (1973) focused on the musculature of *Trigoniophthalmus alternatus*. Klug and Klass (2007) summarized and critically analysed these descriptions.

Despite these contributions, comparative analyses of the abdominal musculature remain challenging. This is largely because homologies for many muscles are unresolved, a situation complicated by the presence in apterygotes (Archaeognatha and Zygentoma) of a system of non-cuticular connective-tissue tendons to which multiple abdominal muscles attach (Bitsch & Bitsch, 2002). In contrast, Pterygota lack these tendons, with muscles inserting directly onto the cuticular body wall. Moreover, each ventral tendon in apterygotes connects to the body wall at several points, making direct comparisons of insertion sites with Pterygota problematic (Kluge & Klass, 2007). The presence of a system of non-cuticular tendons in both Archaeognatha and Zygentoma — a feature present in most non-insect Haxapoda and absent in Pterygota — further underscores the distinctive structural organisation of apterygote musculature (Kluge & Klass, 2007). Collectively, these observations emphasize the evolutionary significance of skeletal muscle architecture in Archaeognatha, highlighting its potential to inform interpretations of insect abdominal evolution.

At the microscopic level, apterygote muscles attach to the cuticle via discrete structures: (1) with junctions between muscle fibers and epidermal cells (myo-epidermal junctions) characterized by regular interdigitations in the contact zone and (2) the epidermo-cuticular junctions. Specialized structures occur in the cuticle at regions of muscle insertion can be observed using the light microscopy (Caveney, 1969). Electron-dense fibers embedded in the procuticle likely enhance the durability of muscle attachments, while the specialized arrangement of these fibers may contribute to the characteristic microsculpture of the cuticular surface. All together, these features illustrate a unique combination of mechanical specialisation and evolutionary retention, distinguishing the skeletomusculature of apterygotes from that of more derived insect groups.

In this study, several regions with clear hexagonal sculpture were observed as imprints on inner cuticle surface of female abdominal ventrums of *Trigoniophthalmus simplex*. These imprints were interpreted as attachment points of the following muscles (compare with Fig 6. from Bitsch, 1974 and Fig. 8 from Bitsch, 1973): endosterno-coxal muscle 47 (Fig. 1, *g*), tergo-coxal muscle 50 with a separate muscle branch 50a, that attached to the (post) laterocoxite (Fig.1, *g*), muscle of stylus 60 (Fig. 1, *b*), and (post) laterocoxal muscle 65 (Fig. 1, *f*). Clear imprint on intersternite 7 (Fig 1, *c*, arrow) may indicate presence of hitherto undescribed medial muscle. It seems to be plausible that these imprints may visualize characteristic junctions between either muscle and cuticle or between non-culicular tendon and cuticle. Nevertheless, whether only skeletal muscles or non-cuticlular tendon too may retain specific imprints on the inner surface of abdominal cuticle in Archaeognatha, has to be explored in further studies.

REFERENCES

Bitsch, J. 1973. Morphologie abdominale des machilides (Insecta Thysanura) — I. Squelette et musculature des segments prégénitaux. *Annales des Sciences Naturelles, Zoologie, Ser. 12*, **15**, 173–200.

Bitsch, J. 1974. Morphologie abdominale des machilides (Thysanura) — II. Squelette et musculature des segments génitaux femelles. *International Journal of Insect Morphology and Embryology*, **3** (1), 101–120. https://doi.org/10.1016/S0020-7322(74) 81010-X

Bitsch, C. & Bitsch, J. 2002. The endoskeletal structures in arthropods: cytology, morphology and evolution. *Arthropod Structure & Development*, **30** (3), 159–177. https://doi.org/10.1016/S1467-8039(01) 00032-9

Caveney, S. 1969. Muscle attachment related to cuticle architecture in Apterygota. *Journal of Cell Science*, **4** (2), 541–559. https://doi.org/10.1242/jcs.4.2.541

Klass, K. D. & Matushkina, N. A. 2012. The exoskeleton of the female genitalic region in *Petrobiellus takunagae* (Insecta: Archaeognatha): Insect-wide terminology, homologies, and functional interpretations. *Arthropod Structure & Development,* **41** (6), 575–591. https://doi.org/10.1016/j.asd.2012.06.003

Klass, K. D. & Matushkina, N. A. 2018. The exoskeleton of the male genitalic region in Archaeognatha, with hypotheses on the early evolution and the morphological interpretation of genitalia in insects. *Arthropod Systematics & Phylogeny,* **76** (2), 235–294. https://doi.org/10.3897/asp.76.e31928

Klass, K. D., Matushkina, N. A. & Kaidel, J. 2012. The gonangulum: a reassessment of its morphology, homology, and phylogenetic significance. *Arthropod Structure & Development*, **41** (4), 373–394. https://doi.org/10.1016/j.asd.2012.03.001

Klug, R. & Klass, K. D. 2007. The potential value of the mid-abdominal musculature and nervous system in the reconstruction of interordinal relationships in lower Neoptera. *Arthropod Systematics & Phylogeny*, **65** (1), 73–100. https://doi.org/10.3897/asp.65.e31666

Lieberman, Z. E., Billen, J., van de Kamp, T. & Boudinot, B. E. 2022. The ant abdomen: The skeletomuscular and soft tissue anatomy of *Amblyopone australis* workers (Hymenoptera: Formicidae*). Journal of Morphology*, **283** (6), 693–770. https://doi.org/10.1002/jmor.21471

Matushkina, N. A. & Klass, K.-D. 2020. Male genitalia of *Charimachilis* (Insecta: Archaeognatha) and the status of archaeognathan "paleoforms". *Organisms Diversity and Evolution*, **20** (2), 253–266. https://doi.org/10.1007/s13127-020-00439-y

Solis, M. A. & Metz, M. A. 2011. Male genital homology based on musculature originating from the tegumen in snout moths (Lepidoptera: Pyraloidea). *Arthropod Structure & Development*, **40** (5), 435–448. https://doi.org/10.1016/j.asd.2011.03.005.

Song, N., Li, X., Yin, X., Li, X., Yin, J. & Pan, P. 2019. The mitochondrial genomes of palaeopteran insects and insights into the early insect relationships. *Scientific Reports*, **9** (1), 1–11. https://doi.org/10.1038/s41598-019-54391-9

Sturm, H. & Machida, R., 2001. *Archaeognatha*. *In*: Handbook of Zoology, vol. 4 (37). Walter de Gruyter, Berlin, 1–213.

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